Review Memorandum

Reference number: 98-1396

Reviewed by: Mary Alice Woody, Ph. D.

Date: 3/13/00

Product: Botulinum toxin type B (BotB) for parenteral use

Sponsor: Elan Pharmaceuticals

Subject: Licensing of manufacturing procedure for botulinum toxin type B (BotB)

Chemistry, Manufacturing, and Controls Section Review

1. Introduction

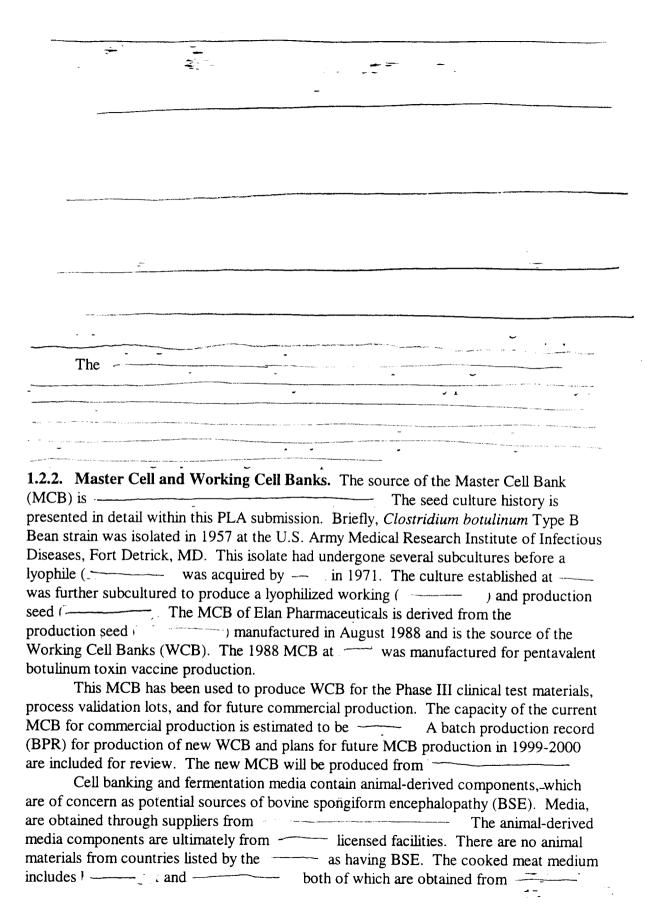
Description of the drug substance and rationale for development. PLA 98-1.1. 1396 has been submitted by Elan Pharmaceuticals for licensing of the commercial production of botulinum toxin type B (BotB) at the San Francisco, CA, facility. Other names of this drug include CAS#93384-44-2 and AN072. There are no nationally approved or pharmacopoeial names. Elan Pharmaceuticals has proposed NeuroBloc as the propietary name for BotB, --- Botulinum toxin type B is intended for parenteral use to induce temporary therapeutic paralysis in muscles.

Botulinum toxin type B is produced during anaerobic fermentation of Clostridium botulinum serotype B. The protein is synthesized as a single polypeptide of and "nicked" by proteases to form the heavy and light chains of ____ and ___ respectively, which are held together by a disulfide bond. Activation accompanies nicking, and extent of nicking correlates with potency. The toxin is associated with several nontoxic proteins that range from ____ to ___ Together with the toxin, the proteins form a complex of about

The botulinum neurotoxins are zinc metalloproteases that bind receptors on neurons, and are internalized via endocytotic vesicles and released to the neuronal cytosol. The toxins cleave proteins, associated with presynaptic docking of exocytotic vesicles containing neurotransmitters, inhibiting the neurotransmitter release. Botulinum toxin type B specifically cleaves at the neuromuscular junction is, resulting in paralysis that gradually reverses.

Several antigenically distinct serotypes of the botulinum neurotoxins exist, among which there is no cross-reactivity of antisera. Botulinum toxin type A is currently used to induce therapeutic muscle paralysis. Some patients that receive multiple treatments with botulinum toxin type A, however, experience reduced efficacy of the drug believed to be related to development of antibodies to the toxin. Antibodies that neutralize type A neurotoxin do not cross-react with BotB. Hence, BotB is potentially an alternative to

activity, extent o	ibutes of the BotB of central concern during drug manufacture are specific, in terms of (U/ml) per concentration of toxin (ng/ml); of nicking, evaluated by complex integrity or consistency, assessed by and size exclusion chromatography; and identity, as established with zing antibodies obtained from the World Health Organization.
materia facility p (Figure been inc	Summary of manufacture of botulinum toxin Type B and evaluation of raw als. Manufacture of BotB concentrated product (CP) at the San Francisco, CA, proceeds through three major steps: fermentation, recovery, and purification 1). Master batch production records for the entire manufacturing process have cluded for review, and these were used for the assistency lots.



reduce the likelihood of contaminatio	n by BSE.	medium is derived from
	_	A study that was
rformed to assess challenges to asepticocess samples, and support of anaerobox. The Elan Pharmaceuticals MCB am stain. The biochemical tests for the ture established August 1988	c processing of the med bic growth was satisfact was characterized by bit the characterization of C.	orily concluded. ochemical profile and botulinum Type B seed
ture purity tests, morphological tests, eterial identification tests have been spunufacturing facility.	Gram stain procedures becified for culture main	, and automated at the Elan
nuracturing facility.		
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		a ana apina matan di padaganaga (dina) o dida din pa di matan kangilan membana dan pagamanan pengahan pengahan

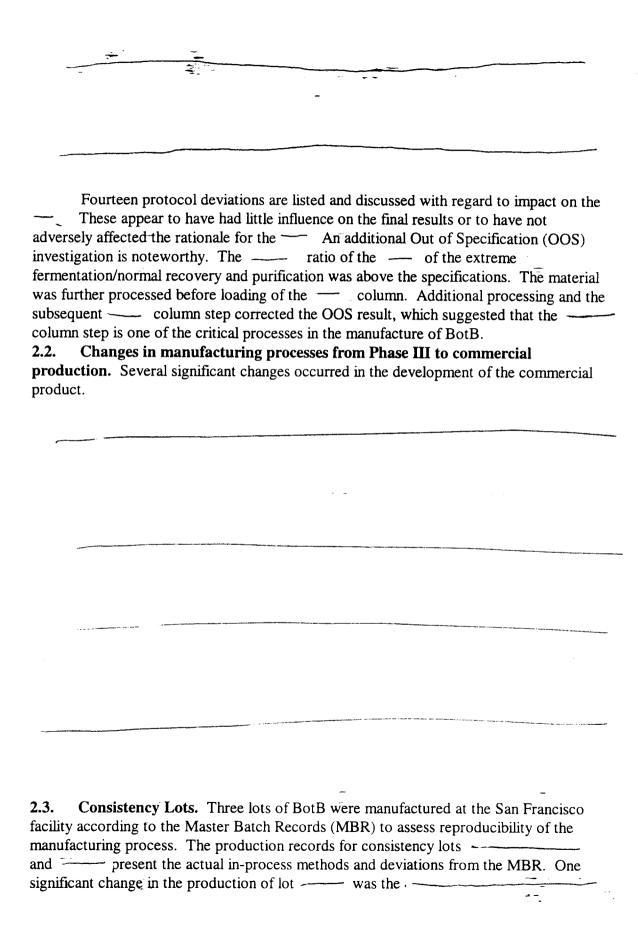
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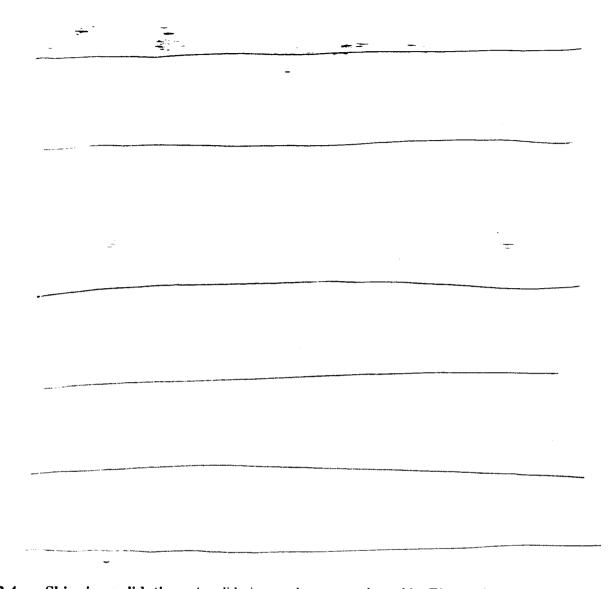
1.3.2. Shipping Procedure. The diluted bulk is shipped to the contract filler in the same — carboy in which it was prepared. The container is packed within a sealed drum with bubble wrap and foam for protection and a temperature recorder to ensure that is maintained. The material is shipped to the contract filler in a dedicated refrigerated truck.

album Mono	The final drug product contains 5000 ± 1000 U/mL of BotB, the active ingredient rug product is additionally formulated with the following excipients: human serum in (HSA), sodium succinate, sodium chloride,, and graphs are provided for all chemicals, which are USP or EP grades, except for the
sodium (HSA) Certifi include three	n succinate (grade not specified). The biological excipient, human serum albumin b, was purchased from are cates of analysis and compliance with Agency regulations for lot are ed. This HSA lot has no reactivity with HBsAg, HIV-1, HIV-2, and HCV. The vial presentations have nominal fill volumes of 0.5, 1, and 2 ml, that provide 2500, and 10,000 U, respectively.
2. 2.1.	Process Development and Validation. Process performance validation.
describ manuf	dertaken at — to assess the reliability of the BotB manufacturing methods bed in the Master Batch Records (MBR) and identify critical steps in the acturing process. In four separate fermentation and purification studies, various acturing processes were altered from the MBR to assess the effects on quality of the
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An Out of Specification (OOS) result that repeatedly occurred in the manufacture of the consistency lots was the ratio that was higher than the
specification. Investigation indicated that the material that increased absorbance at 260 nm was, and the OOS result was ascribed to variable removal of
during a recovery step. The inconsistent content of the preparations was
ultimately attributed to variations during the fermentation. The diafiltration step was extended to reduce the ratio. The downstream ————————————————————————————————————
ratio be used for information only.
2.3.1 Process Validation of Dilute Bulk Solution and Final Fill. CP lot was
used for production of dilute bulk lot — Dilute bulk lots — were
derived from CP lot —— vas not processed further for license
application. Dilute bulk lot —— was used to produce final container lots C95007,
C95008, and C95009. Final container lots C95010, C95011, and C95012 were derived
from dilute bulk lot — Final container lots C95013, C95014, and C95015 were derived from dilute bulk lot —
Process validation for controlled and reproducible production of the dilute bulk
solution, performed by Elan Pharmaceuticals, focused on shipping (discussed below) and
filter validation/recovery. These are well chosen as important, perhaps the critical steps,
of dilute bulk solution preparation and shipment.

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2.4. Shipping validation. A validation study was conducted by Elan to demonstrate that shipment of the dilute bulk solution to the filling facility does not compromise product quality. This study used dilute bulk solutions from the consistency lots and an earlier clinical CP. — was identified as the most critical factor in preservation of product quality. Maintenance of the refrigerated temperature of the cargo area of the dedicated truck and within the shipping drum was monitored during shipment. An illustration of the packing of the — carboy within the stainless steel shipping drum is provided within the submission. The carboy contained — of dilute bulk solution. Absorbent packs and temperature monitors are included in the drum, and the carboy is cushioned against shock with bubble wrap and rubber foam, as possibly excess vibration or shock could cause deterioration in product quality. Shock events were recorded during shipment. Pre- and post-shipment quality control was similar to analytical tests for the dilute bulk solution (listed below under tests for the dilute bulk solution), with the addition of an identity test and elimination of the protein concentration determination.

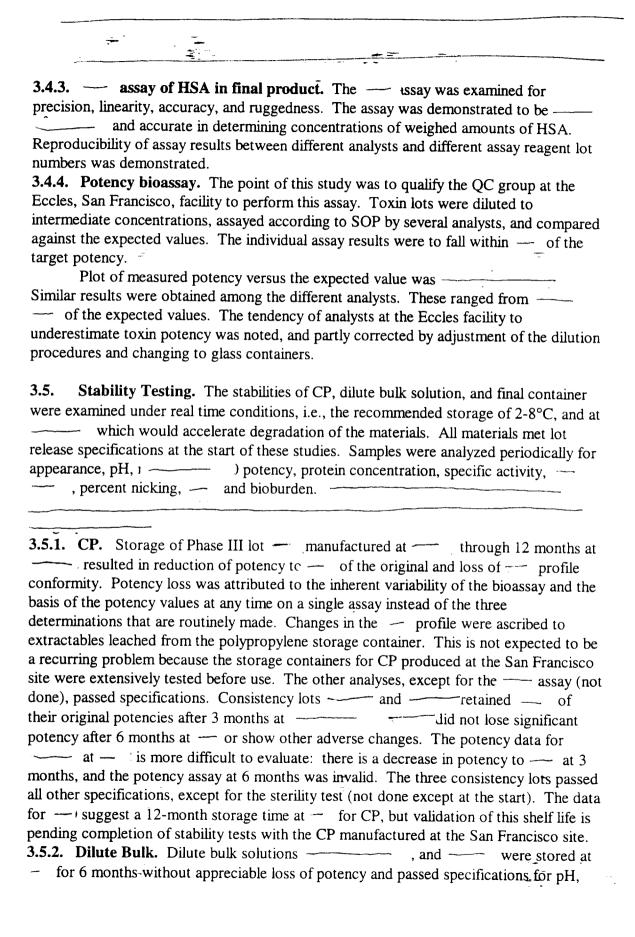
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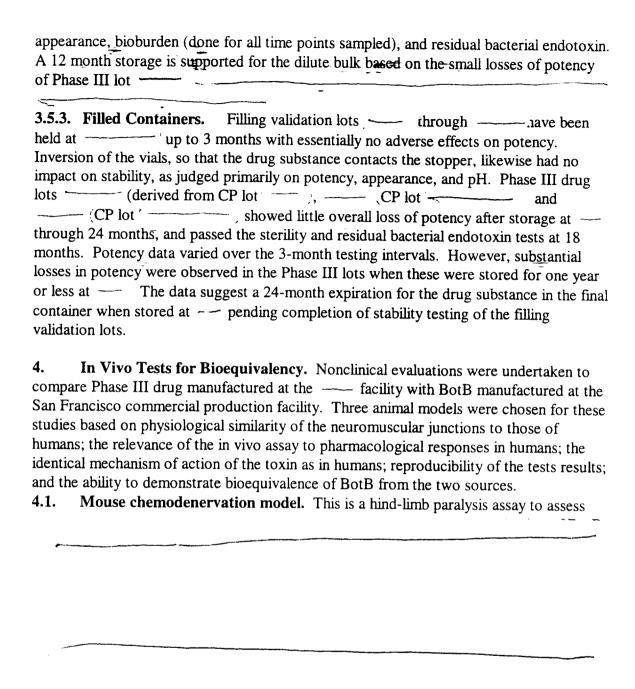
Results indicated that the temperature within the shipping drum could be maintained for the duration of the shipment. The temperature within the drum was — for the four shipments, and the temperature of the cargo area of the truck could be maintained within the specified — The quality of the diluted bulk, as assessed by the analytical tests, was preserved during shipment. No pattern of change of potency was apparent in the data. Appearance, pH, and endotoxin levels were not affected. Microbial contamination of the product during shipment did not occur. Shock and vibration data for each shipment were provided, but the significance of these data is unclear because no correlation of shock with product damage is provided or perhaps is known. Minor deviations from the test protocol were discussed, and process changes were recommended. The most important of these changes was the inclusion of a plastic liner in the drum to maintain outer surface cleanliness of the carboy.

		ave been distributed among Elan, ———ication of some of the contract laboratories to
1.	Elan (San Francisco, CA)	Inactive components; container closure; dilute bulk; final container biochemical and microbiological tests (LAL test)
2.		Container closure; pre-filling bioburden of dilute bulk; final container microbiological tests
3.		Inactive components; final container biochemical and microbiological tests (stability tests)
4.		Inactive components
5.		Inactive components
6.		Dilute bulk (potency test); final container biochemical tests

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Assessment of paralysis was made independently by several observers to compensate for the inherent subjective nature of these measurements.

A preliminary study was conducted to assess the time points for scoring of paralysis and appropriate doses for the definitive study, and to refine methods to make the assay as reproducible as possible. The purpose of the definitive study was to compare BotB manufactured at the San Francisco facility with that manufactured for Phase III clinical trials. Dose response curves for each preparation of BotB were compared and found to be similar in slope and regression equation, suggesting that the toxin from the two sources were bioequivalent.

	Monkey comparability model.
epar	ed at
licat	Electrophysiologic measurements to assess paralysis
licat 3. tent th B	Electrophysiologic measurements to assess paralysis ed that the two BotB preparations were equivalent. Monkey diffusion model. The purpose of these studies was to determine the
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1. How is — which is purchased in carboys, distributed and used to maintain its quality while formulating buffers and cleaning glassware?

a. has been specified at times in PLA 98 1396 (example, p.347, volume 1, for storage and rinsing of a column). Please clarify which grade of water has been in use throughout development and manufacture of BotB.

b. Has the supplier of — been certified?

	for irrigation (WFIr). d. With reference to the — column packing and testing (n. 332 vol. 1) how
	d. With reference to the — column packing and testing (p. 332, vol. 1) how much — was used to rinse the column after cleaning with — and how is cleaning monitored during that process to assure that the — s adequately removed?
2.	Has the human serum albumin (HSA) that has been used for formulating the final product and development of size exclusion chromatography methods for purification of BotB(p. 341, vol. 1) been certified or demonstrated to not be a potential source of blood-borne adventitious agents? a. Was used for testing of the new : column noted in the batch production record for CP lot p. 92, volume 3)? b. Please clarify when use of was stopped and vas substituted as a test of column performance.
3.	What biochemical tests, referred to on page 71 (vol. 1), were performed on the <i>C. botulinum</i> cultures from which the 1988 Master Cell Banks were derived?
4.	Do tests of culture purity include an identity test with specific antisera to demonstrate that <i>the C. botulinum</i> type B Bean culture produces only type B botulinum neurotoxin?
5.	How was the shelf life for the working cell bank determined?
6.	Please clarify the rationale for storage of the MCB in a whereas the WCB is stored
7.	Please clarify whether the of the culture occurs in the fermentor or in another vessel (p. 151, vol. 2, vs. p. 62, vol.1). If the is performed in the stainless steel fermentor, does the fermentor have liner or inner coating that would prevent leaching of metal ions into the culture by the corrosive action of the
8.	Why was no "normal fermentation and recovery/normal purification" performed during the and how were "normal" conditions defined?
9.	Please clarify why the CP release specifications for percent nicking is greater than , whereas the acceptance criteria is (Table 6, p. 159, vol. 2)
10.	How were the various protein assays chosen, and why are different assays used at different points in the drug manufacture?

- Is there a test for identity of the diluted bulk solution? Were there in vitro tests of identity (i.e., Western blots or ELISA with type B-specific antisera) done on the concentrated product?
- 12. Total heterotrophic microbial counts, determined by a pour plate or membrane filtration method, is used as a measure of bioburden.
 - a. Please clarify whether both methods are used on the same samples, or if there are instances in which one test method is preferred over the other.
 - b. Please clarify which organisms, listed on p. 127 of volume 2, are used for the fungistasis/bacteriostasis tests by writing genus names in full.
- 13. Please clarify why the permissible temperature range of the shipping drum, (p. 22, vol, 5), is greater than the range permitted for the truck,
- 14. Please explain the increase in temperature after the end of shipment recorded for dilute bulk batch _____ p.237, and _____ p. 241, vol. 5. Was the product transferred to refrigerated storage before the temperature increase?
- 15. Because filling line no. 2, room 163, is used for other drugs than Neurobloc (p. 137, vol. 4), how is the filling line decontaminated before and after Neurobloc filling to ensure that drugs are not mixed?
- Why are the filled vials that are yet to be inspected (p. 153, vol. 4) held at and not 2-8°C?
- 17. For the media fill of the final product vials, described pages 95-101 volume 5, was an anaerobic test organism used?
- 18. For the sterile media fill described on pages 95-101 volume 5, how was it decided to use

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